《Original Paper》

Body fat mass and nutrient intake in relation to serum leptin levels in female Japanese students

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Summary

Objective:
Leptin is a hormone produced in adipose tissue, regulating appetite. Serum leptin levels are reported to be elevated in obese individuals. Little is known about the relation between serum leptin levels and dietary factors, particularly in Japanese young women. In the present study, we examined the relationship of serum leptin levels to physical atatus, and metabolic abnormalities, and also revealed effects of specific dietary factors on serum leptin levels in female Japanese students.

Methods:
Subjects were 315 female university students, 18-22 years old. Physical measurements and laboratory examinations were performed. Body fat mass was evaluated by body fat percentage, arm circumference (AC), triceps skinfold thickness (TSF), and waist circumference (WC). Serum leptin level was measured by radioimmunoassay (RIA). Intake of macronutrients, vitamins, dietary fiber, and food groups was assessed with a food frequency questionnaire (FFQ).

Results:
Serum leptin levels were significantly correlated with body mass index (BMI), body fat percentage, AC, TSF, and WC. Serum leptin levels were significantly higher in subjects with overweight than in those with normal weight and under weight. BMI, body fat, AC, TSF and WC in subjects with high serum leptin group were higher than those with low and middle serum leptin groups. There were no significant differences in serum blood glucose, HbA1c, total cholesterol, LDL-C, HDL-C, triglyceride and uric acid among low, middle, and high serum leptin groups. There were no significant differences in dietary intake of total energy, carbohydrate, protein, fat, cholesterol, fatty acids, dietary fiber, vitamins and other micronutrients among low, middle, and high serum leptin groups. Higher intakes of soybeans were associated with lower serum leptin concentrations in young Japanese women.

Conclusion:
Serum leptin levels elevated in obese young women, showing a significant correlation with BMI, and the amount of fat stored in adipose tissue. Total energy intake and nutrient composition of diet did not influence serum leptin levels in healthy young women. Higher intakes of soybeans may reduce serum leptin concentration.

Keywords: Leptin, Obesity, food frequency questionnaire (FFQ), Japanese female students

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Introduction

Leptin is a 16-kilodalton adipocyte-derived hormone that circulates in the serum in the free and bound form. Serum levels of leptin reflect the amount of energy stored in adipose tissue[1]. Leptin is produced and secreted predominantly by adipocytes with levels proportional to fat stores and increased by overfeeding. Leptin decreases food intake and body weight while increasing energy expenditure by binding to its receptor in the brain. Obesity promotes multiple cellular processes that attenuate leptin signaling, and amplify the extent of weight gain induced by genetic and environmental factors[2]. However, obese individuals have in most cases, high levels of circulating leptin. The failure of these high levels to control body weight suggests the presence of "leptin resistance" to the hormone that could be partly responsible of disturbances on body weight regulation[3]. Leptin resistance has been considered to be associated with increased body fat and circulating leptin levels. Several investigations revealed that central nervous system mechanisms associated with leptin resistance, but less is known about the role of diet. Recently, specific types of dietary sugars or fats were demonstrated to induce leptin resistance in the absence of increasing serum leptin and body fat mass[4,5]. These findings suggest that specific macronutrients may be involved in the induction of leptin resistance prior to the development of obesity. Diet-induced leptin resistance may play a role in the onset of weight gain leading to obesity.

Appropriate dietary habits encourage maintenance and growth of a body and are also important for the prevention of obesity, underweight, anemia and osteoporosis, especially for young females. Serum leptin concentrations are reported to be correlated with body mass index (BMI) and higher in healthy young women than in age-matched men[6]. Little is known about the relation between serum leptin levels and dietary factors[7, 8]. Murakami K, et al. revealed that intake of dietary fiber, vegetables, and pulses showed an independent inverse association with serum leptin concentration in female Japanese dietetic students[9, 10].

In the present study, we examined serum leptin levels and dietary intake in Japanese female students. We investigated the relation of serum leptin levels to BMI, body fat, and laboratory data reflecting diabetes and dyslipidemia. Also, we revealed effects of specific dietary factors on serum leptin levels.

Subjects and Methods

The present study was approved by the research ethics committee of Nagoya University of Arts and Sciences. All subjects agreed to participate in this study by signing a written informed consent.

Subjects were 315 female university students (18～22 years old). Physical measurements of height, body weight, body mass index (BMI), body fat percentage, arm circumference (AC), triceps skinfold thickness (TSF), and waist circumference (WC), and blood pressure were performed. Fasting blood samples were collected, including measurement of total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, blood glucose, hemoglobin A1c (HbA1c), AST, ALT, γ-GTP, uric acid, creatinine, BUN. Serum leptin level was measured by RIA.

Intake of macronutrients, vitamins, dietary fiber, and food groups was assessed with a food frequency questionnaire (FFQ)[11, 12].

Data are expressed as means ± a standard deviation (SD). The difference of means between more than two groups was assessed by analysis of variance (ANOVA) and Scheffe's procedure for post-hoc analysis. Correlation between two parameters was analyzed by linear regression. Statistical analysis was performed by IBM-SPSS 19.0. $P<0.05$ was considered statistically significant.

Results

1. Serum leptin levels

Serum leptin levels of all subjects were 7.80 ± 3.70 ng/ml (from 1.90 to 24.3 ng/ml), showing a normal distrbution (Fig. 1).

Fig. 1 Distribution of serum leptin levels

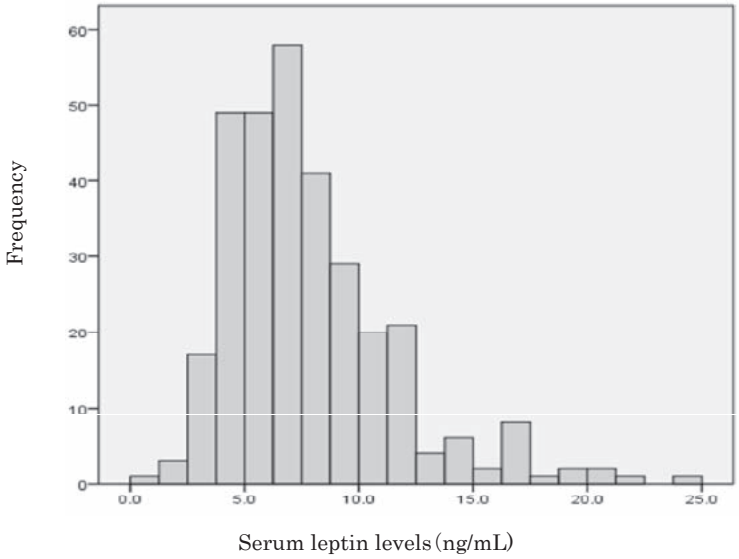

Table 1 Serum leptin levels and BMI

	Body Mass Index (BMI)		
	Low (BMI<18.5) (n=84)	Normal (18.5≦BMI＜25.0) (n=225)	High (25.0≦BMI) (n=6)
Serum leptin levels			
Low (n=110) (<6ng/mL)	39	71	0
Middle range (n=137) (6～10 ng/mL)	36	100	1
High (n=68) (10ng/mL≦)	9	54	5
Mean Serum leptin levels (m±SD) (ng/mL)	6.4±3.4	8.1±3.6＊	13.8±4.7＊#

＊: significant different from subjects with low BMI ($p<0.05$)
#: significant different from subjects with normal BMI ($p<0.05$)

Of all subjects, there were 84 students with underweight (BMI<18.5), 225 students with normal weight (18.5≦BMI<25.0) and 6 students with overweight (25.0≦BMI). Serum leptin levels of subjects with underweight (6.4 ± 3.4 ng/ml) were significantly lower than those with normal weight (8.1 ± 3.6 ng/ml). Serum leptin levels of subjects with overweight (13.3 ± 4.7 mg/dl) were significantly higher than those with normal weight.

Based on the frequency distribution for serum leptin levels of all subjects, we divided the data into 3 groups; low serum leptin levels (<6.0ng/ml, n=110), middle (6.0～10.0 ng/ml, n=137) and high (10 mg/ml≦, n=68).

Frequencies of high levels of serum leptin were significantly higher in overweight group (5/6, 83%) than in normal weight group (54/225, 24%), in underweight group (9/84, 11%).

2. Serum leptin levels and physical status (Table 2)

Serum leptin levels were significantly correlated with BMI (r=0.358), body fat percentage (r=0.421), TSF (r=0.396), AC (r=0.424) and waist circumference (r=0.324), respectively.

Table 2 Serum leptin levels and physical status

	Serum leptin levels			
	Low (<6ng/mL) (n=110)	Middle (6～10ng/mL) (n=137)	High (10ng/mL≦) (n=68)	P values
Body Weight (kg)	47.8±5.6*	49.7±5.3	53.4±8.2#	<0.01
BMI	19.0±1.8	19.8±1.9	21.1±3.0#	<0.01
Body fat percentage (%)	21.9±4.0*	24.8±3.8	26.8±4.5#	<0.01
Arm circumference (AC)(cm)	22.8±2.2*	23.9±1.9	25.3±2.5#	<0.01
Triceps Skinfold (TSF)(cm)	18.8±5.3*	22.1±5.4	25.6±5.7#	<0.01
Waist circumference (cm)	66.3±5.7	67.8±4.9	71.3±6.3#	<0.01
Systolic blood pressure (mmHg)	102±12*	108±11	105±12	<0.01
Diastolic blood pressure (mmHg)	64±9*	67±8	65±8	0.035

*: significant lower than subjects with middle levels of serum leptin ($p<0.05$)
#: significant higher than subjects with middle levels of serum leptin ($p<0.05$)
NS: not significant

Table 3 Serum leptin levels and serum levels of glucose, lipids, and uric acid

	Serum leptin levels			
	Low (<6ng/mL) (n=110)	Middle (6～10ng/mL) (n=137)	High (10ng/mL≦) (n=68)	P values
Fasting blood glucose (mg/dL)	85±13	85±6	86±5	NS
HbA1c (%)	4.9±0.3	4.9±0.3	4.9±0.2	NS
Total choresterol (%)	176±30	182±30	185±31	NS
Triglyceride (mg/dL)	71±32	78±28	78±33	NS
LDL cholesterol (mg/dL)	89±27	89±31	94±31	NS
HDL cholesterol (mg/dL)	64±16	67±18	67±20	NS
Uric Acid (mg/dL)	4.4±0.7	4.4±0.7	4.4±0.9	NS

NS: not significant

Body weight, body fat percentage, AC, TSF and waist circumference in high serum leptin group were significantly higher than in middle serum leptin level group. Also, Body weight, body fat percentage, AC, TSF and waist circumference in low serum leptin level group were significantly lower than in middle serum leptin level group. Systolic and diastolic blood pressure levels in low serum leptin level group were significantly lower than in middle serum leptin level group.

3. Serum leptin levels and blood chemistry (Table 3)

There were no significant differences in serum blood glucose, HbA1c, total cholesterol, LDL-C, HDL-C, triglyceride and uric acid among low, middle, and high serum leptin groups.

4. Serum leptin levels and dietary intake

There were no significant differences in dietary intake of total energy, carbohydrate, protein, fat, cholesterol, fatty acids, and dietary fiber (Table 4).

Table 4 Serum leptin levels and amounts of daily intake of energy, lipid and dietary fiber

	Serum leptin levels			
	Low (<6ng/mL) (n=110)	Middle (6～10ng/mL) (n=137)	High (10ng/mL≦) (n=68)	P values
Total energy intake(kcal)	1701±527	1649±528	1690±448	NS
Carbohydrate(g)	249±73	243±71	247±60	NS
Protein(g)	57.7±21.6	54.8±21.4	55.3±17.3	NS
Fat(g)	49.1±20.2	47.6±20.8	49.5±18.3	NS
Energy ratio				
Carbohydrate(%)	61.0±6.1	61.3±5.8	61.4±5.4	NS
Protein(%)	13.4±1.8	13.1±1.5	13.0±1.5	NS
Fat(%)	25.5±4.8	25.5±4.8	25.8±4.4	NS
Choresterol (mg)	313±183	289±133	303±176	NS
Saturated fatty acid(g)	14.4±6.9	13.9±6.7	14.7±6.5	NS
Monounsaturated fatty acid(g)	16.7±6.9	16.2±7.2	16.7±6.3	NS
Polyunsaturated fatty acid(g)	10.5±4.4	10.0±4.5	10.1±3.6	NS
Dietary fiber (g)	9.2±3.6	9.0±4.3	8.9±3.4	NS

NS: not significant

Table 5 Serum leptin levels and daily intake of dietary fiber

Daily intake of dietary fiber	Serum leptin levels (ng/mL)	Daily intake of dietary fiber	Serum leptin levels (ng/mL)
<6g (n=52)	7.5±3.3	<5g/1,000cal (n=130)	7.8±3.6
6～12g (n=212)	7.9±3.8	5～6g/1,000kcal (n=110)	8.0±3.8
12～17g (n=38)	8.1±3.7	6～7g/1,000kcal (n=45)	7.8±3.7
17g≦ (n=13)	6.4±3.3	7g/1,000kcal≦ (n=30)	7.0±3.5

Table 6 Serum leptin levels and amounts of daily intake of electrolytes, micronutrients and vitamins

	Serum leptin levels			
	Low (<6ng/mL) (n=110)	Middle (6～10ng/mL) (n=137)	High (10ng/mL≦) (n=68)	P values
Potassium (mg)	1782±791	1396±913	1697±723	NS
Calcium (mg)	428±266	406±253	422±261	NS
Magnesium (mg)	192±76	181±78	180±60	NS
Salts (g)	6.5±2.6	6.2±3.3	6.2±2.2	NS
Iron (mg)	6.4±2.6	5.9±2.5	5.9±2.1	NS
Zinc (mg)	7.37±2.48	7.02±p2.40	7.10±2.07	NS
Vitamin A(μg)	407±204	387±231	403±197	NS
Retinol (μg)	220±130	208±156	221±122	NS
Caroten (μg)	2199±1370	2105±1323	2140±1435	NS
Vitamin B1(mg)	0.63±0.27	0.60±0.30	0.60±0.24	NS
Vitamin B2(mg)	0.88±0.46	0.85±0.44	0.87±0.44	NS
Vitamin C(mg)	59.6±37.9	60.5±36.6	56.7±35.7	NS
Vitamin D(μg)	5.11±4.20	4.72±3.51	4.63±2.73	NS
Vitamin E(mg)	5.8±2.4	5.6±2.9	5.8±2.4	NS

NS: not significant

Table 7 Serum leptin levels and daily intake of foods

	Serum leptin levels			
	low (<6ng/mL) (n=110)	middle (6～10ng/mL) (n=137)	high (10ng/mL<) (n=68)	P values
Cereals & potatoes(g)	573±180	547±150	554±150	NS
Fats & oils(g)	17.0±8.5	17.3±9.8	17.5±7.2	NS
Soybeans (g)	47.5±72.4	31.2±31.9	27.5±19.1	<0.01
Fish and shellfish(g)	32.6±26.2	29.4±22.1	30.0±19.5	NS
Meats (g)	51.0±26.9	49.3±31.3	47.5±24.1	NS
Eggs (g)	41.1±37.6	39.7±23.8	41.5±34.4	NS
Daily products (g)	127±175	134±182	146±225	NS
Green vegetables (g)	82.9±80.8	82.5±209.8	80.9±118.2	NS
Other vegetables (g)	79.7±49.6	74.3±44.0	74.0±44.0	NS
Fruits (g)	73.9±66.6	87.7±103.1	69.9±70.7	NS
Confectioneries (g)	143±165	126±189	113±87	NS

NS: not significant

Murakami et al. revealed intake of dietary fiber showed an independent inverse association with serum leptin concentration in a group of young Japanese women[9], so we evaluated the relation between serum leptin level and intake of dietary fiber (Table5). Subjects with large amounts of dietary fiber intake (17.0g/day≦, or 7.0g/1,000kcal/day≦) had lower serum leptin levels than those with lower amounts of dietary fiber intake, but not significantly.

Also, there were no significant differences in dietary intake of vitamins and other micronutrients among low, middle, and high serum leptin groups (table 6).

There were no significant differences in dietary intake of foods among low, middle, and high serum leptin groups (table 6).

Discussion

The physiologic role of leptin has not yet been fully elucidated. The elevation of serum leptin concentration is reported to feature human obesity. Total body fat mass, body fat percentage and BMI are the best predictors of circulating leptin levels[13]. Similarly, age, basal glucose levels, and ethnicity do not influence circulating leptin levels. Diabetes does not influence leptin secretion in both lean and obese subjects. Independent of adiposity, leptin levels are higher in women than in men. This sexual dimorphism is also present in adolescent children[14]. In eating disorders anorexia nervosa and bulimea nervosa, leptin levels are not upregulated but simply reflect BMI and probably body fat[15]. Furthermore, leptin resistance can impair physiological peripheral functions of leptin such as lipid and carbohydrate metabolism and nutrient intestinal utilization[3].

In the present study, we revealed that among female Japanese students, serum leptin level in obese students was higher than that in students with under and normal body weight. We also demonstrated that serum leptin levels were significantly correlated with body fat percentage, TSF, AC and waist circumference, reflecting the amount of subcutaneous and visceral fat storage. Castracane et al[6] reported that serum leptin levels were correlated with BMI and there was no difference in serum leptin levels between young and postmenopausal women. Miller GD et al[16] reported that significant correlations were found between serum leptin concentration and indicators of fat mass in young adult women and men. Nakao, et al[17] reported that serum leptin level correlated with body fat percentage and the ratio of visceral to subcutaneous adipose tissue (V/S ratio). Although *ob* gene is differentially expressed in different fat compartments, apart from total body fat, upper or lower body adiposity or visceral fat does not influence basal leptin levels[13]. Moreover, students with a high level of serum leptin had a high BMI, and body

fat percentage. AC and TSF, reflecting subcutaneous fat mass, and waist circumference, reflecting visceral fat mass, are higher in subjects with a high level of serum leptin than in those with a middle or low leptin level. Obesity and visceral fat deposition increase the risk for metabolic, cardiovascular, malignant, orthopedic and psychological diseases. The role of leptin pathogenesis of those diseases requires further investigation.

Human studies have not shown that leptin concentrations can be changed rapidly by meals[7]. Relatively little is known about the effect of specific dietary factors on circulating serum leptins. Murakami et al[9] demonstrated that higher intakes of dietary fiber, vegetables, and pulses were associated with lower serum leptin concentrations in young Japanese women, independently of potential confounding factors including BMI. Nakamura et al[10] reported that dietary fiber was the only nutrient that had consistently significant inverse relationship with leptin in both men and women.

In the present study, higher intake of soybean was associated with lower serum leptin concentration. Soybean and its isoflavones have been shown to have beneficial effects on carbohydrate and lipid metabolism. Kwak JH et al[18] demonstrated that black soy peptide supplementation was beneficial for body weight control in overweight/obese subjects. Thus, higher intakes of soybeans may reduce serum leptin concentration.

Adiponectin is another adipocyte-derived hormone, the serum levels of which are decreased in obese and insulin-resistant humans. Our previous study[19] revealed that plasma adiponectin levels were significantly lower in obese students than that in students with normal body weight. For nutrients, higher amount of dietary fiber intake was observed in students with higher levels of plasma adiponectin. Also, plasma adiponectin was higher in students with high intake of dietary fiber. However, Murakami et al[20] reported that none of the nutrients and foods was a significant determinant of serum adiponectin concentration. Further studies are necessary for serum adiponectin concentration in relation to nutrient and food in young women, especially obese or lean subjects.

In conclusion, serum leptin levels correlated with BMI and the amount of fat stored in adipose tissue. However, total energy intake and nutrient composition of diet did not influence serum leptin levels in healthy young women. Higher intake of soybean was associated with lower serum leptin concentration.

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要旨

女子大学生の体脂肪分布および食事摂取状況と血中レプチン値の検討

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目的：レプチンは脂肪細胞から分泌され、食欲を調節するホルモンである。血中レプチン値は肥満者で増加することが報告されている。血中レプチン値と食事摂取状況との関連については、特に若い女性では、明らかになっていない。本研究では、女子大学生を対象に、血中レプチン値と肥満度および体脂肪分布との関連を明らかにするとともに、栄養摂取状況と血中レプチン値の関連について検討した。

方法：315名の女子大学生（年齢18～22歳）を対象とした。肥満度や脂肪蓄積状況などの身体所見および糖代謝や脂質代謝に関係する血液検査を実施した。食事調査は食物摂取頻度調査法（FFQ）によって実施した。

結果：血中レプチン値は、BMI、体脂肪率、上腕周囲径、上腕三頭筋皮下脂肪厚、ウエスト周囲径と有意の正の相関を認めた。血中レプチン値は、低体重者や正常体重者と比較して、肥満者では有意に高値であった。血中レプチン値の高値者群では、BMI、体脂肪率、上腕周囲径、上腕三頭筋皮下脂肪厚、ウエスト周囲径および血圧値が、レプチン低値群･中間値群と比較して、有意に高値であった。血液検査では、血中レプチン値の低値群、中間値群、高値群との間で、血糖値、HbA1c 値、総コレステロール値、LDL コレステロール値、HDL コレステロール値、中性脂肪値、尿酸値に有意差は認めなかった。食事調査では、レプチン高値群では大豆類の摂取量が少なかったが、その他、血中レプチン値の低値群、中間値群、高値群との間で、エネルギー摂取量、食物繊維摂取量、栄養素別摂取量および食品群摂取量に有意差は認めなかった。

結論：女子大学生においても、血中レプチン値は肥満者で上昇していた。血中レプチン値は BMI や脂肪蓄積状況を反映する指標と有意の正の相関を示した。食事調査からは、血中レプチン高値群では大豆類の摂取量が少なかったが、他には血中レプチン値に影響を与えるエネルギー摂取状況、栄養素摂取状況、食物群摂取状況は認めなかった。

索引用語：レプチン、肥満、食物摂取頻度調査、女子大学生

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